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*Iowa State University*

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**Order Number 8825474**

**The effects of ionizing radiation on the face fly, *Musca autumnalis*  
DeGeer, irradiated in nitrogen**

**Mansour, Mohammed Youssef, Ph.D.**

**Iowa State University, 1987**

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Ann Arbor, MI 48106

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**The effects of ionizing radiation on the face fly,  
Musca autumnalis DeGeer, irradiated in nitrogen**

**by**

**Mohammed Youssef Mansour**

**A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
Requirements for the Degree of**

**DOCTOR OF PHILOSOPHY**

**Major: Entomology**

**Approved:**

Signature was redacted for privacy.

**In Charge of Major Work**

Signature was redacted for privacy.

**For the Major Department**

Signature was redacted for privacy.

**For the Graduate College**

**Iowa State University  
Ames, Iowa**

**1987**

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## INTRODUCTION

The face fly, Musca autumnalis DeGeer, is native to the old world and has been reported specifically from Europe, Russia, Iran, Palestine, North Africa, China, Korea and Japan (Eltrigham, 1916; Hammar, 1942; Kobayashi, 1919; Okamoto, 1924; Krecob, 1949; Teskey, 1960). In North America, the face fly was first recorded at Middleton, Nova Scotia, in 1952 (Vockeroth, 1953). Since that time, it has spread rapidly west and southward to cover most of southern Canada and northern United States, and by 1973, the fly was present in all of the continental United States except Texas, Florida, New Mexico, Arizona and Hawaii (Pickens and Miller, 1980).

Face flies have sponging mouth parts and feed on the nectar of flowers, manure fluids, saliva, tears, sweat, mucous secretions, and blood and serum oozing from wounds made by other insect bites (Hammar, 1942; Teskey, 1969). On cattle and horses, face flies congregate around the nose and eyes to feed on mucous secretions. When the flies congregate around these areas, the animals become nervous, stop feeding, group together head first in an attempt to keep the flies off, or seek protection in shady places. The reduction in feeding and grazing time, and the energy spent in fighting and kicking, have been reported to cause decrease in weight gain and reduction in milk production (Peterson and Boreherding, 1962; Hansens, 1963; Wrich, 1970). In addition to its effects on cattle and horses, the face fly may move into suburban areas where it may annoy perspiring humans and create a nuisance by overwintering in their homes (Singh et al., 1966).

Because of the fly's habit of feeding on the mucous secretions of the eyes of cattle and horses, it is a good mechanical vector of the bacterium

Moraxella bovis (Haudurug), the causative agent of pink eye (Hall, 1984). The face fly is also a true biological vector of at least four species of Thelazia eye worms (Krecob, 1949; Steve and Lilly, 1965), and Parafilaria bovicola (Tubangui), a filarial nematode that causes cutaneous bleeding of cattle (Steen et al., 1982).

The annual losses in control costs and production losses due to face flies are estimated to be in excess of 150 million dollars (Anonymous, 1976), and it is considered to be a major pest of livestock in the United States. The face fly is primarily a pest of cattle; however, it has also been reported to be a pest of bison (Burger and Anderson, 1970), horses (Teskey, 1960), yaks (MacNay, 1961), sheep and deer (Teskey, 1960), and even of humans (Dobson and Matthew, 1960).

Although the face fly is quite susceptible to most of the insecticides commonly used for livestock pests, chemical control methods for this insect are inadequate because of its habit of feeding on the faces of cattle, an area extremely difficult to treat, and its ability to disperse widely from the area of its origin (Pickens and Miller, 1980). Current control techniques include self-treating devices (dust bags and oilers), sprays, and ear tags, all of which have limited effectiveness.

Biological control of the face fly has been considered. Attempts to rear several hymenopterous parasites, which have been used with some success in controlling other dipteran species, have been unsuccessful because they were not able to emerge from the heavily calcified face fly puparia (Hair and Turner, 1965; Burton and Turner, 1968; Thomas and Wingo, 1968; Hayes and Turner, 1971). Aleochara tristis Gravenhorst is a

coleopteran that preys upon the face fly in France (Drea, 1966). Releases of this beetle in the United States established populations in four locations in Nebraska and Montana, but effects on face fly populations have not been evaluated (Jones, 1971). Heterolylenchus autumnalis Nickle, a nematode parasite of the face fly, has been highly effective in reducing face fly populations. Reported levels of parasitism of face flies by this nematode vary from a low of 3% in Ontario (Wright, 1972) and 19% in Iowa (Krafsur et al., 1983) up to 40% in central Missouri (Thomas and Püttler, 1972). The nematode may be a promising biocontrol agent, but much work remains to be done in this area. Bacillus thuringiensis was used as a feed additive and found to be effective in reducing face fly larval development, (Hower and Cheng, 1968), but no recent pathogenic studies have been conducted with it.

Several insect growth regulators have been tried and shown to kill face fly larvae in manure pats (Miller, 1974). However, destruction of other arthropods within the manure pat makes its application of questionable value (Blume et al., 1974). These materials are also of little use to individual producers because adults can migrate from neighboring premises in sufficient numbers to maintain damaging adult populations on herds (Kunz et al., 1972).

The lack of practical biological and chemical control of the face fly, in addition to the hazards of treating dairy and beef animals with certain insecticide formulations (Claborn et al., 1960), has prompted the consideration of genetic methods to control or eradicate this pest.

Metcalf and Metcalf (1982) defined genetic control as: ". . . .a variety of methods by which a pest population can, in theory or practice, be controlled through the manipulation of its genetic components or other mechanism of inheritance" Genetic manipulation of insect pests is a potentially attractive method of control because of its specificity. The numerous types of manipulation fit into two categories: (1) The release of partially sterile or fully fertile insects that carry inherited factors which will affect several subsequent generations. This category includes chromosomal translocations, recessive lethal mutations, sex-limited lethal mutations, detrimental and conditional lethal mutations, and a combination of meiotic drive plus detrimental or lethal factors, (2) The release of sterile insects that affect only the succeeding generation. This category includes hybrid sterility, cytoplasmic incompatibility and sterile males.

The sterile male release method has certain advantages over other genetic techniques. For example, there is no need to rear a strain that has a high degree of sterility, and no concern that the accidental release of incompatible females along with males might result in the establishment of a nonindigenous strain. The theoretical basis of the sterile male release technique was summarized by Knipling (1955). He wrote that if sterile males were released in numbers equal to those of the males in the wild population, both would have an equal opportunity of inseminating wild females, provided the sterile males were equally competitive. Therefore, the number of insects in the ensuing generation would be reduced by approximately 50%, since only half the number of eggs laid could hatch. Knipling's (1964) models showed that by flooding each succeeding generation

of a natural population with a fixed number of sterile males, equal to nine times the number of wild males in the original population, complete eradication could be achieved in four generations.

In his review of the sterile insect release method, Christenson (1966) stated that the practicality of the sterile male concept was initially established when the screw-worm fly Cochliomyia hominivorax (Coquerel) was eradicated on the island of Curacao and then throughout its range in the southeastern United States. Knipling (1964) has enumerated nine basic requirements which must be considered in determining the feasibility of utilizing the sterility method for insect population control:

1. Availability of a method of inducing sterility without serious adverse effects on mating behavior and competitiveness.
2. A method of mass-rearing the insect involved.
3. Quantitative information regarding population densities at the low level in the population cycle.
4. A practical way of reducing natural populations to levels manageable with sterile insects.
5. Information on rate of population increase as a guide for determining the numbers of sterile insects required to overflow the natural population.
6. Cost of current methods of control plus losses incurred by the insect pest must be higher than the combined cost of initial reduction of the natural population and rearing, treating, and releasing the required number of sterile insects.



7. If complete population control cannot be maintained because of reinfestations by migrating insects or new introductions, then the cost of maintaining complete control by continual sterile insect releases must be favorable in relation to the costs for current methods of control plus additional losses caused by the insects.
8. The sterile-insect release method of control might be justified if it overcame the hazards to man or his environment of other control methods.
9. Sterile insects to be released must not cause undue losses to crops or livestock, and must not create hazards for man which might outweigh the benefits obtained by achieving and maintaining population control.

The face fly is an insect which may prove to be suitable for control by the sterile insect method: It can be mass reared (Arends and Wright, 1981); some information regarding population dynamics is available (Pickens and Miller, 1980); it has sufficient economic importance to require continual and costly control programs (Anonymous, 1976); and the number of sterile insects required to overwhelm a natural population would be least in the spring when the natural population is lowest.

One of the most important prerequisites for the success of the sterile male release method is the development of techniques that will sterilize both sexes without seriously affecting male behavior, particularly mating ability and longevity.

One approach to producing sterility in insects is by the use of chemosterilants. Several chemosterilants have been shown to be effective in sterilizing face flies (Kaur and Steve, 1969; Hair and Turner, 1966).

However, the lack of an efficient method to apply these chemicals and the high mammalian toxicity of some of them have prevented their practical application under field conditions.

Sterility in insects may also result from ionizing radiation. Successful control of other dipterous insects using ionizing radiation led to my previous investigation of its effect and potential use against the face fly (Mansour, 1984). That study showed that exposing six day old pupae to 1600 rads of gamma radiation induced 97.3% sterility in face fly males and completely prevented ovarian development in the females. However, this dose also reduced male survival and competitiveness.

Reduced longevity in male insects exposed to ionizing radiation has been one of the most commonly observed responses. Although the cause of reduced longevity caused by ionizing radiation is not well understood, some explanations have been advanced. For example, in the adult boll weevil, early death was explained by the effect of radiation on mid-gut epithelium, where radiation inhibited mitosis and prevented the replacement of degenerated cells (Riemann and Flint, 1967). Irradiation may also shorten the life span of an insect by increasing its susceptibility to attack by microorganisms (Jafri, 1964). There is also evidence that certain protozoan parasites in the fat body destroy the fat after irradiation, causing reduced longevity (Jafri, 1964).

The sexual competitiveness of a sterilized male insect depends on male longevity, general activity, sexual aggressiveness, and the type of sterility induced. The type of sterility (aspermia, inability to mate, sperm inactivation and dominant lethal mutations) depends mainly on the

dose used, stage of development, and oxygen level at the time of irradiation.

One of the principal requirements for the success of the sterile male release method is the production of healthy, competitive (though genetically different) males and their ability to live as long as natural males. Because of the critical importance of these two components (longevity and competitiveness) to the success of a sterile male release program, further research is needed to examine the possibility of inducing complete male and female sterility in the face fly without affecting male survival and competitiveness.

One suggested method of maintaining fitness of radiosterilized males is by irradiating as late as possible in the life cycle and by reducing the dose to the minimum level required to cause an acceptable level of sterility. Since irradiation done during the last day before emergence can be postponed only a few hours, delay may produce little improvement. Reducing the dose to less than 1600 rads may not be desired either, because this may also reduce the level of sterility. However, at this point, it should be pointed out that sterility has been assessed on the basis of percentage egg hatch, and dominant lethal mutations induced by radiation could also be expressed at later stages of development (Borkovec, 1966; Smittle, 1967). Therefore, if sterility is measured as percentage pupation or pupal emergence, lower doses might prove to be useful.

Another method of retaining vigor in sterilized male insects is by irradiating under anoxic conditions. Nitrogen and carbon dioxide have generally been used successfully in improving longevity and/or

competitiveness of radiosterilized males (Hallian and Rai, 1973; Baumhover, 1963; Hooper, 1971; Smittle, 1967; El-Gazzar et al., 1983; Earle et al., 1979; Zumreoglu et al., 1979; Baldwin and Chant, 1971).

The purpose of my investigation was to obtain data regarding the possibility of sterilizing the face fly without adversely affecting its mating behavior or other fitness components by irradiating in a nitrogenous atmosphere. The specific objectives were to determine:

1. The effects of irradiation under anoxic conditions on pupal emergence.
2. The effects of irradiation under anoxic conditions on fertility and fecundity.
3. The survival of the  $F_1$  generation during larval and pupal stages.
4. The effects of irradiation under anoxic conditions on male longevity.
5. The competitiveness of sterilized males.
6. The degree of permanence of male and female sterility.
7. The mating habits of the female face fly under laboratory conditions.

## REVIEW OF THE LITERATURE

## Biology of the Face Fly

Understanding the biology and ecology of the face fly is important for the development of control measures. The face fly has been the subject of research since it was introduced into North America in 1952 (Vockeroth, 1953). Studies have dealt largely with its population dynamics, diapause, laboratory rearing, mating habits and conventional methods of control (Pickens and Miller, 1980). These studies have added greatly to our knowledge of the bionomics and laboratory rearing of this species. No attempt will be made here to review the literature completely. However, several facets of the biology of the face fly are related directly to my investigations and will, therefore, be briefly reviewed.

The adult stage

Nomenclature In his original description of the face fly, DeGeer, 1776, named the species Musca autumnalis. Its classification as presented by Teskey (1960) is:

Order: Diptera

suborder: Cyclorrapha

division: Schizophora

section: Calyptratae

family: Muscidae

genus: Musca

species: autumnalis DeGeer

Four subspecies of M. autumnalis have been described: autumnalis DeGeer, pseudocorvina Van, somalorum Bezzi, and ugandae Van. The typical subspecies (autumnalis) is the form found in most of the Palaearctic Region (Europe, Palestine, Kashmir, and China) and in North America. The other three subspecies are confined to the Ethiopian Region (Aberdare Range, Kenya; Somaliland; Birunga, Uganda) (West, 1951).

Feeding Hammer (1942) considered that M. autumnalis is one of a group of flies that are in transition between general secretion suckers and blood suckers. He reported that M. autumnalis feeds on the nectar of a wide variety of flowers and suggested that nectar was the principal diet of the flies before cattle were pastured in the spring. However, during the breeding season, face flies feed mainly on fluids on the surface of dung, the mucous secretion from the eyes and noses of cattle and horses, and on the blood or discharge exuding from wounds (Hammer, 1942; Teskey, 1969). Although the flies have been reported to prefer dark or spotted cows, (Hansens and Valiela, 1967; Ode and Matthysse, 1967; Engroff et al., 1972), Teskey (1969) did not find any fly preference for any cattle breed, color, or age. Feeding occurs only during daylight hours (Hammer, 1942). The majority of flies feeding on dung and cattle secretions are females, while males are seldom noted on cattle (Teskey, 1969). Flies on dung are generally gravid while flies with immature ovarioles are found more often on cattle (Kaya and Moon, 1980).

Mating habits The literature concerning mating habits of the face fly is somewhat confusing. Hammer (1942) reported that conspicuous objects in the field, such as a lone bush, a rock, or a cow, were the sites of

mating. He observed that males resting on such objects darted out and copulated with females that were flying past. Wang (1964) observed a laboratory colony and found that females mated either on the sides of the cage or on the ceilings and that mating flights described by Hammer rarely occurred. Males start to mate 2-5 days after emergence while females commence mating at 3-5 days of age (Wang, 1964). Males were reported to mate an average of four times (Lodha et al., 1970. Teskey (1960) found that females mated only once in laboratory cages, and Lodha et al. (1970a) reported that sperms were stored in the spermatheca for 28 days. Contrary to Teskey's findings, Wang (1964) reported that some females copulated 2-3 times with different males.

Oviposition Female face flies begin to lay eggs 2-5 days after mating (Wang, 1964). Fresh dung is the primary and possibly the only oviposition site of the face fly. There is a preovipositional period of 4-12 days, and ovipositional peaks at 6-11 days of adult age, depending on the ambient temperature (Heller, 1976; Miller, 1967). Maximum oviposition occurs on dung 20 minutes old, but dung remains slightly attractive to adult flies for as long as 24 hours (Hammer, 1942). Eggs of laboratory-bred flies are laid in batches which average 18-20 eggs (Wang, 1964). Batches of eggs are usually produced every 3-4 days at 25°-30°C (Wang, 1964). In the laboratory, the total number of eggs produced by a single female ranges from 30-128 (Wang, 1964). However, in the field, the number of eggs laid by a single female is likely to be much less, depending on her longevity (Krafsur et al., 1983).

Longevity In laboratory studies, Turner and Hair (1966) reported that adult face fly longevity decreased with an increase in the temperature. The average adult longevity under different temperature regimes in cages reported by these authors was 5.5 days at 38°, 7 days at 32°, 11.5 days at 27°, and 17.5 days at 21°. The life cycle of the face fly under pasture conditions usually requires about 17-21 days (Hammer, 1942; Wang, 1964) and adult females live an average of 9.6 days (Krafsur et al., 1983).

Dispersal In general, face flies are most active on bright, warm days when winds are less than 16 km/h. Flight begins when the ambient temperature is 17°C and the flies are most active at temperatures between 25-29°C provided the light is suitably intense (Hammer, 1942; Hansens and Valiela, 1967; Ode and Matthysse, 1967; Teskey, 1969; Peterson and Meyer, 1978). Dispersal between pastures is unaffected by time of day or fly age if the environmental conditions are optimal (Ode and Matthysse, 1967). Dispersal studies showed that face flies move 0.5-11 km over a period of 3-5 days (Fales et al., 1964; Killough and McClellan, 1965; Hansens and Valiela, 1967; Ode and Matthysse, 1967). However, the spread of the face fly over most of North America in less than two decades may have been caused by distribution of flies over long distances as cattle were transported from one area to another.

Diapause Studies of diapause induction in the laboratory showed that the critical scotophase for inducing diapause in the face fly was 15 hours of darkness at 16°C (Caldwell and Wright, 1978). The same authors found that diapause termination was gradual and successful only if the



flies were exposed to four months of total darkness at 5°C. In the field, photoperiods and temperatures existing in late August and early September may cause adult face flies less than 2 days old to enter diapause (Caldwell and Wright, 1978; Stoffolano and Matthysse, 1967). Like some Culex and Anopheles mosquitoes (Washino, 1977), face flies destined to diapause develop an extensive fat body (Stoffolano and Matthysse, 1967), and both sexes become photophobic until they become active again in mid to late April (Ode and Matthysse, 1967). Overwintering females have undeveloped ovaries and are uninseminated. Overwintering males possess mature testes with motile spermatozoa (Ode and Matthysse, 1967). Diapause termination in the field is nearly synchronous and usually occurs in mid to late April. Church steeples, houses, barns, and hollow trees are all reported to be the overwintering sites for diapausing face flies (Benson and Wingo, 1963; Strickland et al., 1970). Although face flies have been reported to overwinter in specific structures year after year (Caldwell, 1975; Matthew et al., 1960), Strickland et al. (1970) reported that color and function were not important factors in choosing the overwintering sites; however, tall buildings were apparently preferred to others.

Population dynamics      If effective control of an insect pest is to be accomplished, it is important that one obtain as much data as possible concerning the population dynamics of the species. Only by studying the seasonal fluctuations in population density, the reproductive capacity of the species, and its population growth rate will one be able to select the most effective control measures to employ, to time properly their applications, and to evaluate accurately the degree of success. This is

especially true when one is considering the use of the sterile male release method for population control.

Several investigators have studied the seasonal occurrence and abundance of face flies. In Denmark, Hammer (1942) described the seasonal fluctuation of face fly numbers on cattle as bimodal. He reported that in Denmark, the first peak counts occurred in late April to early May, presumably the result of overwintered flies. Then, the population started to decline until the first summer generation of flies was produced. The second peak observed by Hammer (1942) occurred in early June as first generation flies emerged. The maximum abundance of flies on cattle occurred in late July and early August. During late August and early September, face fly populations decreased in number, presumably the result of flies entering diapause and going to overwintering sites (Ode and Matthyse, 1967). In North America, face fly populations usually peak in late May and early August. Then, the population starts to decline in late August and early September as flies enter diapause and go to their overwintering sites (Caldwell, 1975; Ode and Matthyse, 1967).

#### The egg stage

The eggs of the face fly are yellowish-white, with a long, grayish-black respiratory mast at the anterior end (Hammer, 1942; Teskey, 1960; Wang, 1964). The eggs are positioned in dung so that the respiratory masts are exposed to the air. Under laboratory conditions, incubation of the eggs requires 21-22 hours at 20°C, and 9-10 hours at 35-40°. No hatch occurs at 11°C (Wang, 1964).

### The larval stage

Face flies have a typical muscoid larva (Wang, 1964). The three instars feed in the dung on the products of fermenting organic materials. First stage larvae stay in the upper layer of the dung, while the other two instars go down to the bottom (Hammer, 1942). Larval development is temperature dependent, and requires from 13 days at 13.8°C, to 2.54 days at 34.1° (Moon, 1983); no larval development occurs at 0°C (Wang, 1964). The optimal rearing temperature for face fly larvae has been reported by Killough and McClellan (1965) to be  $27 \pm 1^\circ\text{C}$ .

Egg and larval stages have been reported to have high mortality. In the field, the combined egg and larval mortality was estimated to be 50-90% (Wang, 1964; Teskey, 1969; Valiela, 1969; Burton and Turner, 1968). In the laboratory, however, the combined mortality has been estimated to be 21-26% (Killough and McClellan, 1965). The high mortality of the prepupal stages in the field is caused by predators attacking eggs and, especially, the first instars (Valiela, 1969).

### The pupal stage

Full-grown third instars crawl out of the dung and pupate in the soil or under organic debris surrounding the dung pat (Hammer, 1942; Jones, 1968). Usually pupation takes place within two to seven meters of the pat, depending on weather and the amount of surrounding vegetation (Jones, 1968). According to Moon (1983), pupal development requires from 33.15 days at 13.8°C to 5.7 days at 34.1°. The optimum temperature for pupal development is 29°C (Turner et al., 1966), and no emergence occurs at a constant temperature of 11°C (Wang, 1964). Emergence of adults from the

puparia occurs mainly in the early morning. Emerged flies need at least one hour to complete the transformation and to become fully active (Hammer, 1942; Wang, 1964).

#### Insect Radiosterilization and Some Radiobiological Principles

Although the mutagenic effects of radiation and chemicals have been known since early in this century (Morgan and Runner, 1913; Muller, 1927), for years little was done to develop the concept of practical use of the mutagenic factors for insect pest control. Understanding the effect of mutagenic agents on living organisms requires some knowledge about these agents and their mode of action. The wide variety of mutagens are grouped broadly into three classes (Burns, 1976): (1) temperature shock, (2) chemicals, and (3) radiation.

The last class is divided into two categories, nonionizing and ionizing radiation, according to the effects on living cells. The latter is further divided into particulate radiation, such as alpha and beta particles, and electromagnetic radiation, such as gamma radiation and x-rays (Martin and Harbison, 1979).

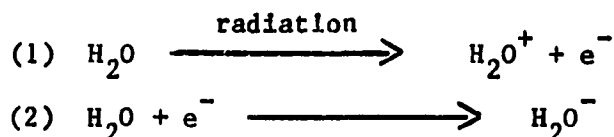
Particulate radiation possesses mass and consists of either helium nuclei (alpha radiation) or nuclear electrons (beta radiation). Electromagnetic radiation, on the other hand, consists of packets of energy transmitted in the form of wave motion (Pizzarello and Witcofski, 1975).

Ionizing radiation affects living cells by transferring its energy to some of the molecules of which cells are made. Energy transference takes place when radiation interacts with some of the electrons in the orbits of some of the molecules. The interaction between radiation and an orbital

electron results in either an ionization or an excitation. Ionization is the removal of an orbital electron from its atom. Excitation, on the other hand, is the elevation of an orbital electron to an orbit more distant from the nucleus. The difference between the two processes (ionization and excitation) depends on the amount of absorbed energy. However, both ionized and excited states are unstable and have a great potential for chemical reactions (Martin and Harbison, 1979).

Martin and Harbison (1979) consider that the ionization process leading to radiation damage requires four stages. The following summary is abstracted from their discussion.

1. The physical stage. The energy is deposited in the cell and causes ionization. In water, the ionization process may be written as:



2. The physico-chemical stage. The unstable ions ( $\text{H}_2\text{O}^+$ ,  $\text{H}_2\text{O}^-$ ) are believed to interact with other water molecules, resulting in new products,  $\text{H}^+$ ,  $\text{OH}^-$ ,  $\text{H}^\bullet$  and  $\text{OH}^\bullet$ . The first two ions ( $\text{H}^+$ ,  $\text{OH}^-$ ) are present in water and probably take no part in subsequent reactions. The other two products ( $\text{H}^\bullet$ ,  $\text{OH}^\bullet$ ) are called free radicals. Free radicals each have a single unpaired orbital electron and are chemically highly active. The other important reaction product produced in this stage is hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). Hydrogen peroxide is a strong oxidizing agent and is poisonous to living cells if present in sufficient quantities.

3. The chemical stage. Free radicals and oxidizing agents may attack the complex molecules which form the chromosomes, causing chromosomal changes in the treated cells.
4. The biological stage. Chromosomal changes caused by free radicals and oxidizing agents may cause cell death, prevent mitosis, or produce heritable modifications that are passed on to daughter cells.

Radiation effects are dependent upon radiation dose. Two units, the roentgen and the rad, have been used to express radiation dose. The roentgen (r) is a unit used to express the amount of ionization caused in air by x-ray and gamma radiation. The rad, on the other hand, is a measure of energy deposition per gram of tissue for any type of ionizing radiation (Pizzarello and Witcofski, 1975).

The magnitude of radiation effects on living cells depends on two kinds of factors. Intrinsic factors depend on the state of the irradiated cell itself and extrinsic factors depend on the radiation and surrounding environment. No attempt will be made here to review the subject fully. However, several facets are related directly to my work and are, therefore, summarized. Much of the following summary is abstracted from Pizzarello and Witcofski (1975), Martin and Harbison (1979) and O'Brien and Wolfe (1964).

A. Extrinsic factors:

1. Radiation dose: Radiation effects depend on the dose accumulated in the body. The dose itself depends on the dose rate and the amount of time the body was exposed to radiation.

2. The kind of radiation: All forms of ionizing radiation have the same biological effect (O'Brien and Wolfe, 1964). However, there are some variations in penetration power and ability to deposit energy. One comparison was made by Amy (1955) using beta, gamma, and x-rays against haploid eggs of the braconid wasp Habrobracon juglandis (Ashmead). These studies showed that gamma rays were the most effective and x-rays the least effective.
3. Dose administration: A given dose is usually more effective when given at one time than when divided into smaller doses with intervals between them. In insects, a study on Drosophila larvae showed that a dose of 6925 r at a rate of 540 r per minute caused 57% mortality after pupation. However, a higher dose (7000 r) at a rate of 78 r per minute caused only 39% mortality (Villée, 1946).
4. Temperature: A study on the hymenopterous parasite Dahlbominus fuscipennis (Zett.) showed a major effect of temperature upon sensitivity to radiation (Baldwin and Narraway, 1957). For instance, a dose of 200,000 r of x-rays given to adult females 24-48 hours after emergence at 10-25°C, caused 30% mortality. However, at 35°C the mortality was 100%.
5. Oxygen, nitrogen and carbon dioxide: Ionizing radiation produces mutations by both direct and indirect effects. The formation of free radicals by the indirect effect, and their action on the genes is probably as important as the direct effect. Since a cell is composed mostly of water, much of the indirect effect will be the action of radiation on water molecules to produce free radicals, thereby

initiating chain reactions (Pizzarello and Witcofski, 1975). Oxygen enhances somatic effects of radiation by interacting with radiation-produced free radicals to produce auto-oxidative chain reactions and to promote the formation of hydrogen peroxide. Changing the composition of a gas mixture will effect a change in oxygen tension at the tissue or cellular level of a respiring organism. The fact that reduced oxygen tension decreases damage from ionizing radiation has been known for some time (Thoday and Read, 1947). Both nitrogen and carbon dioxide have been shown to have a protective action on insect pupae during irradiation (Baumhover, 1963; Smittle, 1967; Hooper, 1971; Hallian and Rai, 1973; El-Gazzar et al., 1983; Earle et al., 1979; Zumreoglu et al., 1979; Baldwin and Chant, 1971).

B. Intrinsic factors:

1. Age and stage of life cycle: There is a general relationship between age, stage of development of the irradiated organism and radiation sensitivity. Generally, radiosensitivity decreases as age increases. Radiosensitivity changes little throughout adult life. During periods of rapid growth, organisms are most radiosensitive. This sensitivity is related to differential cellular activity and degree of tissue differentiation.

In insects, egg and larval stages are relatively susceptible to radiation, but immediately after the onset of pupation, resistance to radiation increases. The gradual increase in radioresistance with age of the pupae has been described in many insect species (Ramsamy, 1977; Nair, 1962; Bushland and Hopkins, 1953). By the time the pharate adult



is visible in the puparium, cell division and differentiation have largely been replaced by cell enlargement and decreased radiosensitivity. At this time, cell division still occurs in the gonads and these are easily damaged by radiation. This explains why the sterilizing dose differs enormously from the lethal dose.

2. Sex, species and chromosome number: The sensitivity of insects to ionizing radiation through the life cycle is dependent not only on the species, but also on the sex and chromosome number. In mammals, females are generally more resistant to radiation than males. Insects are very similar, with male insects in most species more sensitive to radiation effects than females. For instance, in the American cockroach Periplaneta americana irradiated with 10,000 rads, females survived about 14 days, but the males lived only 8 days (Garney, 1959). Haploidy and diploidy have been studied by Clark (1961) as factors that influence radiosensitivity. His study on the braconid wasp Habrobracon juglandis showed that diploid males were less sensitive to radiation than haploid males.

#### Population Control by the Sterility Method

##### The origin of the sterile insect concept and its practicality

The sterile insect release method is the term used to describe the concept of control in which sexual sterility is used to control insect populations. Entomologists have known for many years that insects could be sterilized by exposure to x-rays or gamma radiation. Runner (1916) observed that cigarette beetles, Lasioderma serricorne (Fab.), produced

infertile eggs after exposure to x-rays. Muller (1927) found that mutations could be induced in Drosophila by exposure to x-rays. However, despite Runner's suggestion that x-rays be used to treat tobacco infested with cigarette beetles, nothing was done to develop a practical method of insect control by sterilization.

The idea of introducing sterile males into natural populations of screw-worm flies to achieve control was proposed informally to colleagues by Knipling about 1938 (Lindquist, 1955). Subsequent studies by Bushland and Hopkins (1951, 1953) demonstrated that screw-worm pupae were easily sterilized by x-rays or gamma radiation. They showed that sterile males mated normally with females that subsequently laid infertile eggs. The success achieved in eradicating the screw-worm fly, first on the island of Curacao (Baumhover et al., 1955) and then throughout its range in the southeastern United States (Bushland, 1960; Knipling, 1960) proved the practicality of the sterile insect technique.

In addition to the eradication of the screw-worm fly, the sterile release method has been used successfully against natural populations of other species. These species include the oriental fruit fly, Dacus dorsalis (Hendel), the melon fly Dacus cucurbitae (Coq.), (Christenson, 1966) and the codling moth Cydia pomonella (L.), (Proverbs, 1982). Eradication of Culex quinquefasciatus Say from a small island by release of chemosterilized males was nearly achieved. However, the program was discontinued because of unfavorable weather conditions (Patterson et al., 1970).

### Types of induced sterility in insects

According to LaChance (1967), induced sterility in insects may be due to any of four principal causes.

Dominant lethal mutations In insects, the term "dominant lethal mutation" was coined by Muller (1927) in the same paper in which he reported the discovery of mutagenic effects of radiation in Drosophila. Sonnenblick and Henshaw (1941) defined dominant lethal mutation ". . . nuclear alterations which can effect the death of the zygote even though they are present in a single dose, that is introduced by but one of the germ cells which unite at fertilization."

Dominant lethal mutations can be induced in germ cells of insects by chemicals and by electromagnetic radiation as well as by particulate radiation (Baker and Von Halle, 1954; Clark et al., 1957). Radiation-induced dominant lethal mutations arise as a result of chromosome breaks in the treated cell (Muller, 1940; Pontecorvo, 1941; LaChance, 1967). This kind of mutation is referred to by geneticists as chromosomal mutation or so-called "chromosomal aberrations." Chromosomal aberrations may take several forms (deletions, inversions, translocations and duplications) and result in alterations in the amount or position of genetic material (Burns, 1976). However, dominant lethals also can be due to point mutations (LaChance, 1967). Point mutations, as defined by Burns (1976), are changes within the DNA polymer. These changes may be due to the direct and indirect action of ionizing radiation on DNA synthesis or the result of a base-change or base deletion that alters the base sequence of the molecule (Pizzarello and Witcofski, 1975).

Chromosome changes induced by radiation result in acentric fragments and dicentric chromosomes, which in turn cause chromosome imbalance during cleavage in the zygote. The cytological changes usually cause death. In insects, death due to dominant lethal mutations generally takes place in the embryo prior to egg hatch, usually during the earliest embryonic divisions before blastoderm formation (Von Borstel, 1955; LaChance and Rieman, 1964). However, death may occur in any stage of development (Borkovec, 1966).

The frequency of dominant lethal mutations induced by radiation is proportional to the dose (LaChance, 1967). Moreover, most of the studies on radiosterilization among insects show that the curves relating dominant lethals to radiation dose are not completely linear (Sonnenblick, 1940; Catcheside and Lea, 1945; Fano and Demerec, 1941; Fano, 1947; LaChance and Crystal, 1965). These studies show that dominant lethals are related linearly to low doses. At higher doses, the frequencies of dominant lethals increase asymptotically to unity. Thus, the curve relating dominant lethals to radiation dose consists of a linear and a nonlinear component. At low doses, the linear relation of dominant lethals to radiation dose is most simply explained in terms of a single break or point mutation in the chromosome (single-hits) (Muller, 1954), followed by sister-strand fusion and bridge formation during the first cleavage division. At higher doses, however, the lack of linearity may be due to numerous breaks in the chromosome (multiple hits). Consequently, the nonlinear part of the curve is explained by the increasing frequency of multiple-hit events at higher doses, and since these are superfluous for

the induction of dominant lethals, the curve approaches the maximum gradually.

Sperm inactivation      A second cause of sterility in male insects could in theory be due to the inactivation of the sperm when males are exposed to radiation or chemosterilants, evidenced by loss of sperm motility, inability of sperm to penetrate the egg, or by functional failure in the early stages of embryogenesis. LaChance et al. (1967) considered sperm inactivation an undesirable method when the females are polygamous, because further matings with fertile males would completely negate the effect of previous sterile matings. However, they did point out that sperm inactivation might be effective in those instances where females are inseminated only once and sperm transfer is not required for monogamy.

Aspermia      Aspermia can be defined as the failure of a male to produce sperm. LaChance et al. (1967) suggested that aspermia would be desirable only under those instances where, again, the female mates only once and sperm transfer is not required for monogamy.

Infecundity      The fourth explanation for induced sterility in males, reviewed by LaChance (1967), is infecundity. It is possible that, although mating occurs between a sterile male and a normal female, sperm may not be transferred. Unless this female mates again with a normal male, all eggs oviposited by her would fail to hatch. Further, Borkovec (1966) and others have demonstrated that certain chemosterilants can cause retardation or complete cessation of ovarian development in house flies. The net result is infecundity. LaChance and Bruns (1963) reported that exposure to gamma radiation retarded ovarian development in the screw-worm fly. LaChance

(1967) felt that this form of sterility may be important in the control of populations by the sterility method.

LaChance (1967) and LaChance et al. (1967) considered the merits of each of these four types of male sterility when used in mass release programs. They concluded that sterility due to dominant lethal mutations in the sperm was the most desirable.

#### Methods of inducing sterility in insect populations

There are three basic techniques currently used to induce sterility in an insect population. In the first technique, insects may be mass-reared, radio-sterilized, and released into a natural population to achieve control. Secondly, chemosterilants may be applied to a field population, causing it to destroy itself. Thirdly, strains of insects which are genetically incompatible or which produce hybrid sterility may be released to produce sterility in a natural population. This latter technique has not been studied as a means to control face fly populations. It is possible that strains of face flies from other parts of the world might be incompatible with American strains. This might be a very promising area for future research.

As indicated above, chemosterilants can be used as a substitute for radiation in sterilizing insects for future releases. Smith et al. (1964) pointed out that chemosterilants are generally more economical to use than radiation, and frequently cause less somatic injury to treated insects. Chemosterilants could be used to their greatest advantage, moreover, in treating a portion of a natural insect population in situ. Smith et al. (1964) pointed out that treatment of natural populations would make it

unnecessary to rear and release large numbers of insects to achieve population control. This would substantially reduce costs of control programs. Moreover, some economically important insects cannot be reared in large enough numbers for release programs. With other species, radlosterilized, released insects may themselves be dangerous, destructive, or annoying (e.g., stable flies, mosquitoes). Smith et al. (1964) further pointed out that the chemical induction of sterility in a large proportion of the natural population would be highly advantageous, especially if sterile males mated with the untreated females that escaped the chemosterilant.

As previously discussed, laboratory and field studies have been conducted to determine the effect of chemosterilants on the reproduction of many species of insects. Several studies have evaluated their effect on face flies. The results of these studies will not be fully discussed here. Suffice it to say that several compounds, including apholate, boric acid, hempa and metepa, cause varying degrees of sterility in male and female face flies. For complete results of chemosterilant tests against face flies, the reader is referred to Hair and Adkins (1964), Hair and Turner (1966), Dorsey (1967), Kaur and Steve (1969), and Lang and Treece (1972).

Despite the hypothetical advantages of chemical sterilization of insects, certain disadvantages limit their use. The most important limitations are their mutagenic properties and their high mammalian toxicities. Fahmy and Fahmy (1964) discussed the genetic hazards to man, and the toxicology of chemosterilants has been reviewed by Hayes (1964). He stated that the most promising chemosterilants are known to be acutely

toxic to warm-blooded animals at relatively small doses. The use of chemosterilants is further limited by the development of resistance in certain insects. Resistance has been selected in the laboratory in Aedes aegypti (Linn.) and in Musca domestica Linn. (Hazard et al., 1964; Klassen and Matsumura, 1966; Morgan et al., 1967).

The use of chemosterilants in insect control is further limited by two additional factors. Not all chemosterilants penetrate sufficiently to produce 100% sterility in both sexes. Further, in their review, Graham and Harris (1966) pointed out that sterilizing doses of many chemosterilants also produce adverse effects on survival, longevity, mating aggressiveness, or on other characteristics of the treated insects. Because of these disadvantages, research has continued into the use of radiation-induced sterility in insects.

The theoretical basis of the sterile male release method and the use of x-rays and gamma radiation to induce sterility in insects prior to their release in a sterile male release program was previously discussed. Most of the previously cited examples of successful sterile male release used this technique to induce sterility.

#### Radiation Sensitivity of the Face Fly

Studies of the face fly have largely dealt with its biology, laboratory rearing, diapause, control using conventional methods, and the effects of chemosterilants on its reproduction and longevity. Little work has been done on the effects of radiation on the face fly.

The results of cytological studies (Tung et al., 1970) indicated that 2 and 4 kilorads of gamma radiation applied to the pharate adults 96 hours



after the onset of puparium formation resulted in a progressive decrease in the number of spermatogonia and spermatocytes undergoing premeiotic DNA synthesis. The only additional study on the effect of ionizing radiation on the face fly is that of Gregory and Wright (1973). They stated that a dose of 2.5 krad of gamma radiation given to 5-day old face fly pupae produced complete sterility in both sexes. However, they made no mention of the effect of gamma radiation on male survival or competitiveness. My previous study on the effects of gamma radiation on face fly pupae (Mansour, 1984) showed that exposing 6-day old pupae to 1600 rads of gamma radiation induced 97.3% sterility in face fly males, based on egg hatch and completely prevented ovarian development in the females. However, this dose also adversely affected male survival and competitiveness.

In this regard, it should be noted that some hazards exist when percent egg hatch is the sole criterion used to determine the degree of sterility produced. Borkovec (1966) stated that a number of chemosterilants fed to adult house flies have a moderate to no effect on egg hatch but the larvae fail to reach the pupal stage. A similar situation might also occur when certain insects are exposed to dosages of ionizing radiation. Smittle (1967) exposed house flies to gamma radiation. His data demonstrated that, at a given dosage level, percent sterility based upon egg hatch varied somewhat from the percent obtained when percent pupation was used as the basis for computation.

## METHODS AND MATERIAL

Maintenance of Musca autumnalisSource

The face flies used in these investigations came from a colony that originated from flies collected at several locations around Ames in the summer of 1985. The colony was maintained in a small rearing room in the insect physiology laboratory at Iowa State University. Face flies from a local natural population were periodically introduced into the colony during the summer of 1986.

Laboratory conditions for rearing

Adults for the stock colony and the experimental flies were maintained in a mass-rearing room. Several colonies of the American cockroach, Periplaneta americana, were also reared in the same room. The temperature was maintained at  $29 \pm 1^\circ\text{C}$ . Lighting was controlled by an automatic time clock on a 16:8 (L:D) cycle, and the temperature was recorded with a thermograph. No attempt was made to control the humidity except for several plastic dishes filled with water.

Maintaining adult flies

Adult flies were maintained in wooden cages (32 x 32 x 30 cm) with three screened sides and the fourth covered with wood. The wood piece had a 15 x 26 cm rectangle cut in the middle, to which a cotton sleeve 50 cm long was attached. The top of the cage was screened, while the bottom was a removable board. These cages are referred to as stock colony cages.

Each cage was supplied with water in two glass vials closed with clean cotton balls. Flies were fed on a dry diet consisting of one part powdered egg, one part powdered sugar, and two parts nonfat powdered milk. Powdered egg was included in the diet to maximize egg production (Arends and Wright, 1981). Diet was provided in plastic petri dishes (1 cm deep and 8.5 cm in diameter). Food and water were checked every day and resupplied whenever necessary. Approximately 200 flies were kept in each cage. The cages were placed randomly on the colony shelves, and a 100-watt light was installed above each cage. Cages were cleaned with a wet sponge after each use.

#### Collecting eggs, rearing larvae and handling the pupae

For oviposition, ca. 200 g of fresh cattle dung was placed in a small plastic oviposition dish (surface area ca.  $46 \text{ cm}^2$ ). Four dishes of dung were placed into each stock colony cage in the morning and removed in the evening. The dishes of dung containing eggs were incubated at  $28^\circ\text{C}$  for 24 hours to permit hatching. After the eggs had hatched, the dung from the oviposition dishes was transferred to bigger plastic larval rearing dishes (14 x 14 x 2.5 cm) filled with cattle dung. The manure containing the newly emerged larvae was carefully placed, oviposition-side up, on the fresh manure to prevent death of larvae by anoxia. The amount of fresh dung was determined by the number of eggs present, large numbers of eggs being divided among several larval rearing dishes. If the dung was too dry when collected, water was mixed with it to prevent larval loss through desiccation.

Larval rearing dishes were placed in pupation trays, 55 x 50 x 4 cm, provided with a 1 cm deep layer of fine sand on the bottom. Trays containing the larval rearing dishes were incubated at 28°C. When mature, larvae usually crawled off the dung and dropped into the sand, where they pupated. The pupae were left in the sand until they were two days old to complete calcification, after which they were separated by sifting through a metal screen. After separation, the pupae were counted and put in small plastic dishes, 8 x 8 x 2 cm, with a layer of 1 cm of fine moistened sand on the bottom. The pupae were incubated at 28°C and moistened every day to prevent desiccation. One day before emergence, which was seven days after pupation, dishes containing the pupae were placed in an emergence container and maintained under bright light. Two days after emergence of the first adult, flies were transferred to the stock colony cages. Carbon dioxide was used to anesthetize the flies during the transfer.

#### Testing Procedures

##### Irradiation of pupae and handling of adult flies

The first three radiation treatments were made in a cobalt-60 gamma radiation facility located at the Nuclear Engineering Laboratory, Iowa State University, Ames, Iowa. Because that facility closed temporarily for technical reasons, the rest of the treatments were made with a similar cobalt-60 machine at the Mary Greeley Medical Center, Ames, Iowa.

Six-day-old pupae were irradiated in glass vials (8 cm long x 2 cm diameter). Vials containing pupae were placed in an insulated container for transportation between the entomological laboratory and the irradiation

source in order to minimize temperature fluctuation. The following radiation doses were used: 500, 1000, 1500, 2000 and 2500 rads.

To provide anoxic conditions during irradiation, nitrogen was circulated for 10 minutes in the six vials containing the pupae. Nitrogen was introduced through a duct in the vial lid with a multisample nitrogen distributor. After that, the tubes were sealed with plastic tape and irradiated 15-30 minutes later.

Irradiated pupae were returned to the laboratory and placed individually into small plastic dishes (8 x 8 x 2.5 cm) containing 1 cm fine moistened sand. Dishes containing pupae were appropriately labeled and placed separately in emergence cages. Emergence cages containing irradiated pupae were put under bright light for adult eclosion.

The flies were sexed within 12 hours of emergence. Carbon dioxide was used to anesthetize flies. Seventy-five flies from each sex were put in separate experimental cages (35 x 21 x 24 cm) and the same number of unirradiated flies of the opposite sex were added. Experimental cages were placed randomly on the colony shelves under the same conditions of light and temperature, and fed on the same diet as used for the stock colony.

#### Studies of radiation effects on adult eclosion

During these investigations, the effects of gamma radiation dose on eclosion of face fly pupae irradiated in nitrogen were studied. Five replicates were used in this experiment, and five groups of untreated pupae served as controls.

Emergence cages were examined every morning until adult eclosion ceased. Numbers of unmated males and females eclosing were recorded to

determine the ratio of males to females, as well as the effect of radiation on adult eclosion. Eclosion was expressed as a percent of the number of adults successfully eclosing to the total number of pupae used in the test. The flies were then used in the studies on fertility, as explained below.

Studies of radiation effects on reproduction and survival of the  $F_1$  generation

In these tests, the effects of radiation on reproduction were investigated. Treated and untreated virgin males and females were obtained as described in the previous section. Twelve experimental cages were, therefore, used in each experiment. The caged flies were maintained at a mean temperature of  $29 \pm 1^\circ\text{C}$ .

Six days after the crosses were made, two plastic dishes (8 x 8 x 2.5) containing about 200 g of fresh cattle dung were introduced to each experimental cage between 8 and 9 a.m. and removed between 5 and 6 p.m.

The plastic dishes containing the eggs were incubated at  $28^\circ\text{C}$  and after 24 hours examined under a binocular microscope at 25 diameters magnification. The number of eggs laid and the number of eggs hatched were recorded, and this procedure was repeated 3-4 days later. Ten females from each treatment group were dissected in 0.75% NaCl solution and the three spermathecae were removed, dissected in 0.75% NaCl solution and examined for the presence of spermatozoa under a microscope at 200 diameters magnification. The dung containing the hatched eggs was transferred into larval rearing dishes as before. The larval rearing dishes were placed in pupation boxes (30 x 19 x 12 cm) containing a 2 cm layer of fine sand. Pupation boxes containing the larval rearing dishes were incubated at  $28^\circ\text{C}$ ,

and survival to the adult stage was recorded by counting the number of pupae collected from each tray and the number of flies that emerged from these pupae. This general procedure enabled me to examine the effects of radiation on the fecundity of treated females, the ability of treated males to inseminate normal females, radiation effects on induction of dominant lethal mutation in gametes, and  $F_1$  survival to the adult stage. Percent fertility was determined on the basis of egg hatch, pupation, and pupal emergence.

#### Studies of the effects of radiation on longevity

It is of practical importance that insects which are sterilized and released into a wild population for control purposes should survive nearly as long as those in this population. In most of the radiation-sterilization studies cited earlier in this dissertation, the longevity of sterilized males was considered an important factor in the success of a sterile male release program. Unless a given treatment produces a sterilized male which survives long enough and is sexually competitive enough to mate normally with wild females, then this treatment is only of limited value in a sterile male release program.

Because of this, it was considered essential that some preliminary data regarding longevity in irradiated male face flies be obtained. Experiments were, therefore, performed to determine the effect of increasing dosages of gamma radiation upon face fly males irradiated as 6-day old pupae in nitrogen. In these studies, the longevity of each male used in the sterility experiment was determined. The experimental cages were examined

every other day for 24 days. Dead flies were removed and their number and sex recorded.

Studies of the effects of radiation on male competitiveness

Knipling (1964) enumerated nine basic requirements which must be considered in determining the feasibility of utilizing the sterility method for insect population control. The first of these stated that a method must be available to induce a high level of sterility in the insects without severely affecting longevity. Not only must this method produce relatively long lived, sterile insects, but it must accomplish this without adversely affecting mating behavior and competitiveness. Sterile males which are unable to successfully compete with normal males for normal females in the natural population are unsatisfactory for use in a sterile insect release program. For this reason, it was considered essential that mating competitiveness tests be included in these investigations.

One method for examining competitiveness in the laboratory is by using ratio tests. In this procedure, irradiated males that had received 2500 rads in a nitrogenous atmosphere were confined with untreated males and untreated virgin females in different ratios (0:1:1, 1:0:1, 1:1:1, 9:1:1); each ratio was replicated three times. Seven and 11 days later, eggs were collected from each cage as before, incubated at 28°C for 24 hours, and egg hatch determined.



### Studies on the degree of permanence of male sterility.

Previous experiments on the effects of radiation on reproduction showed that male and female face flies were sterilized completely by a dose of 2500 rads. However, these results did not show how permanent that sterility was. Therefore, experiments were conducted to determine whether or not there was any return of fertility in treated flies subjected to multiple matings over a period of 21 days. Flies emerging from pupae exposed to 2500 rads were segregated by sex into experimental cages. Newly emerged unirradiated males or females were added to make the sex ratio in each cage 1:1. Fertility was examined after six days, as previously described. After oviposition on day seven, females in the cage which had the irradiated males were killed and replaced by newly emerged virgin females equal to the number of males remaining alive. Fertility of the females was examined six days later and the same procedure was repeated a third time. Ten females from each trial were dissected to determine if insemination had occurred. In this experiment, four replicates were used and 200 pupae were irradiated in each replicate. Four groups of untreated flies were used as controls.

### Studies on the mating habits of the female face fly

The literature concerning mating habits of the female face fly includes conflicting reports. Teskey (1969) reported that face flies mate only once, while Lodha et al. (1970a) reported repeated matings. The number of matings is an important factor in a sterile male release program, especially if the sperm transferred by sterilized males are not as motile as those produced by normal males. In females that mate more than once,

sperm immotility would not prevent the production of fertile eggs with subsequent matings (Curtis, 1968). Because of that, it is important to determine whether or not the female face fly mates more than once.

To examine this hypothesis, two experiments were conducted. First, normal females were confined for six days with the same number (75) of sterilized males exposed to 2500 rads as six-day old pupae. After that, female fertility was examined, as previously described, and sterilized males were replaced with normal males of the same age and equal in number to the number of females still alive. Six days later, female fertility was examined again and changes in percentage fertility were recorded. This experiment was replicated four times.

In the second experiment, 10 virgin females were labeled with a small dot of nail polish applied on either the right or left side of the mesothorax. Five colors were used (beet, cherry, red, pink and lilac), and the marked females were confined with an equal number of virgin males. When the flies were two days old, they were checked every 45-60 minutes during the light period for mating pairs until they were seven days old. The sexes were separated at night. Mating females were identified and recorded; this experiment was repeated four times.

## RESULTS

The Effect of Gamma Radiation Under Anoxic Conditions  
on Adult Eclosion

The effect of increasing dosages of gamma radiation under anoxic conditions upon eclosion in face flies irradiated as 6-day old pupae is summarized in Table 1. The results indicate that none of the radiation dosages consistently reduced the percentage of adult eclosion. To test this, an analysis of variance was performed according to the method of Snedecor and Cochran (1957). A randomized block model was employed, where replicates were treated as blocks. The ANOVA (Table 2) shows no significant difference between any treatment or control group at the 0.05 probability level.

Studies on the Effects of Gamma Radiation Under  
Anoxic Conditions on Reproduction

The effects of gamma radiation on reproduction in face flies were evaluated. Radiation effects on fecundity in females and fertility in both males and females were determined.

The effects of gamma radiation on female fecundity

The effects of gamma radiation on fecundity was investigated by recording the number of eggs laid by each treatment group. The data are presented in Table 3. The results indicate that none of the radiation dosages caused a consistent decrease in egg production. The analysis of variance (Table 4) shows no significant difference between treatment and control groups at the 0.05 probability level.

Table 1. The effect of gamma radiation under anoxic conditions on face fly pupal emergence<sup>a</sup>

Radiation dose in rads	Number of pupae treated	Number of emerging adults	% Eclosion
0	1000	935	93.5
500	1000	900	90.0
1000	1000	932	93.2
1500	1000	902	90.2
2000	1000	919	91.9
2500	1000	922	92.2

<sup>a</sup>Data are the sum of five replicates.

Table 2. Randomized block analysis of variance to determine the effects of gamma radiation on adult eclosion of face flies

Source of Variation	D.f	Sum of squares	Mean squares	F	P
Replicate	4	217.7	54.4	3.46*	>0.025
Treatments	5	216.3	43.3	2.75	>0.05
Error	20	314.7	15.7		
Total	29	748.7			

\*Significant at the 5% level of probability.

Table 3. The effects of gamma radiation under anoxic conditions on female face fly fecundity<sup>a</sup>

Radiation dose in Rads	Total number of eggs laid	Mean number of eggs/replicate $\pm$ standard deviation
0	2178	435.6 $\pm$ 63.7
500	2376	475.2 $\pm$ 47.09
1000	2403	480.6 $\pm$ 28.82
1500	2115	423.0 $\pm$ 43.02
2000	2214	442.8 $\pm$ 22.11
2500	2193	438.6 $\pm$ 45.50

<sup>a</sup>Data are the sum of five replicates.

### The effects of gamma radiation on fertility in face flies

Insect sterility may be due to either one or a combination of the following factors: (1) aspermia or lack of egg production, (2) sperm inactivation, (3) the inability to mate, and (4) dominant lethal mutations.

Experiments were conducted to determine the relationship between radiation dose and fertility, and to evaluate the factors contributing to the sterility.

Male fertility      Male fertility was measured as percentage egg hatch, pupation, and pupal emergence from crosses of treated males to untreated females. The relationship between radiation dose and male sterility is shown in Table 5. It is clear that egg hatch, as well as pupation and pupal emergence, decreased with increasing radiation dose. A dose of 2500 rads reduced egg hatch to less than 1% and caused 100% mortality in the larvae which were able to hatch, as no pupae were collected from these treatments. It is also clear from the tables that the effect of radiation on fertility decreased proportionally with increasing radiation dose. While a dose of 1500 rads reduced egg hatch to about 10%, a further 500 rads was required to lower egg hatch to about 2% and another 500 rads to reduce egg hatch below 1%. A consistent decline in percentage pupation and pupal emergence from those hatched eggs or pupated larvae also occurred. For example, while 89.8% of the hatched eggs in the controls gave pupae, only 44% of them did so for the flies which were exposed to 2002 rads, and none of them did so in the 2500 rads treatment. A similar trend occurred in percentage emergence.

Table 4. Randomized block analysis of variance to determine the effects of gamma radiation on female face fly fecundity

Source of Variation	D.f	Sum of squares	Mean squares	F	P
Replicate	4	3420.5	855.13	.395	>0.25
Treatments	5	20359.8	4071.96	1.88	>0.1
Error	20	43236.7	2161.84		
Total	29	67017			

The data in Figure 1 were derived from egg hatchability studies of normal females crossed to treated males. On the basis of this study and other studies in the literature (LaChance and Crystal, 1965; Lee, 1958; Proverbs and Newton, 1962), it appears that the curve relating sterility response to radiation dose is a curvilinear one over a wide range of doses. Figure 1 shows that the decrease in fertility follows a linear relation at low doses; however, at high doses fertility increases less rapidly.

Since the relationship between radiation dose and sterility response is a curvilinear one when plotted in arithmetic units (Figure 1), it is often difficult to compare results from different laboratories and with different species. However, if gamma dose is plotted in arithmetic units and percent egg hatch as a logarithmic transformation, a linear relationship is obtained (Figure 2). Figure 2 shows a very good linear relationship between radiation dose ( $x$ ) and log percent fertility response ( $y + 1$ ). The strength of this relationship is confirmed by a large correlation

Table 5. The effects of gamma radiation under anoxic conditions on male face fly fertility<sup>a</sup>

Radiation Dose in Rads	The criteria used for measuring sterility						
	% Egg Hatch			% Pupation		% Pupal Emergence	
	Total Number of eggs laid	Total Number of eggs hatched	% Hatch	Total Number of pupae collected	% Pupation	Total Number of pupae eclosed	% Emergence
0	2353	2165	91.6	1945	82.7	1762	74.9
500	2396	1295	54.0	1108	46.2	986	41.2
1000	2152	525	24.4	412	19.2	346	16.1
1500	2445	249	10.2	185	7.6	147	6.0
2000	2430	55	2.3	23	.95	14	.6
2500	2348	18	.8	0	0	0	0

<sup>a</sup>Data are the sum of five replicates.



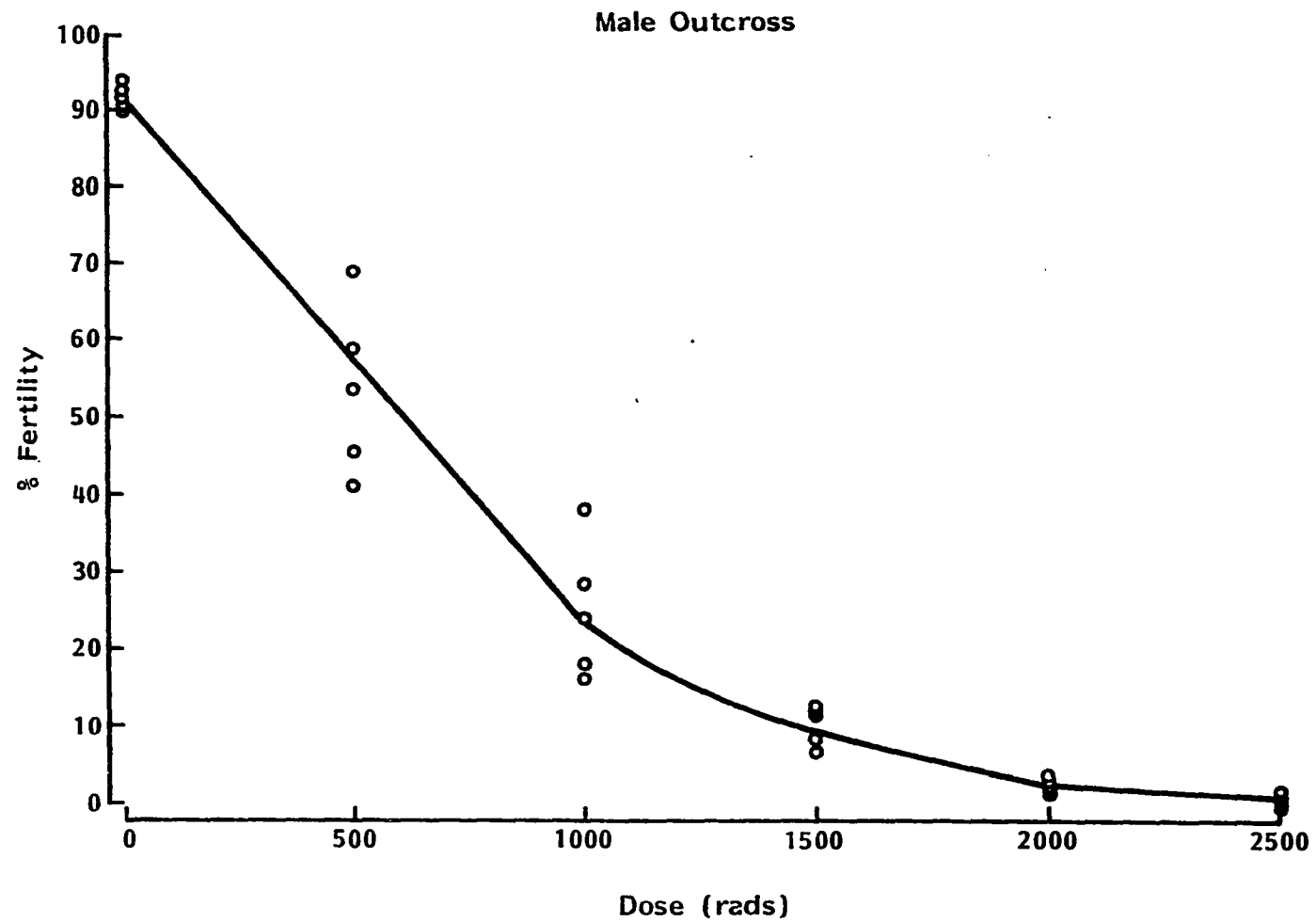


Figure 1. Effect of increasing gamma ray dosage on face fly male fertility

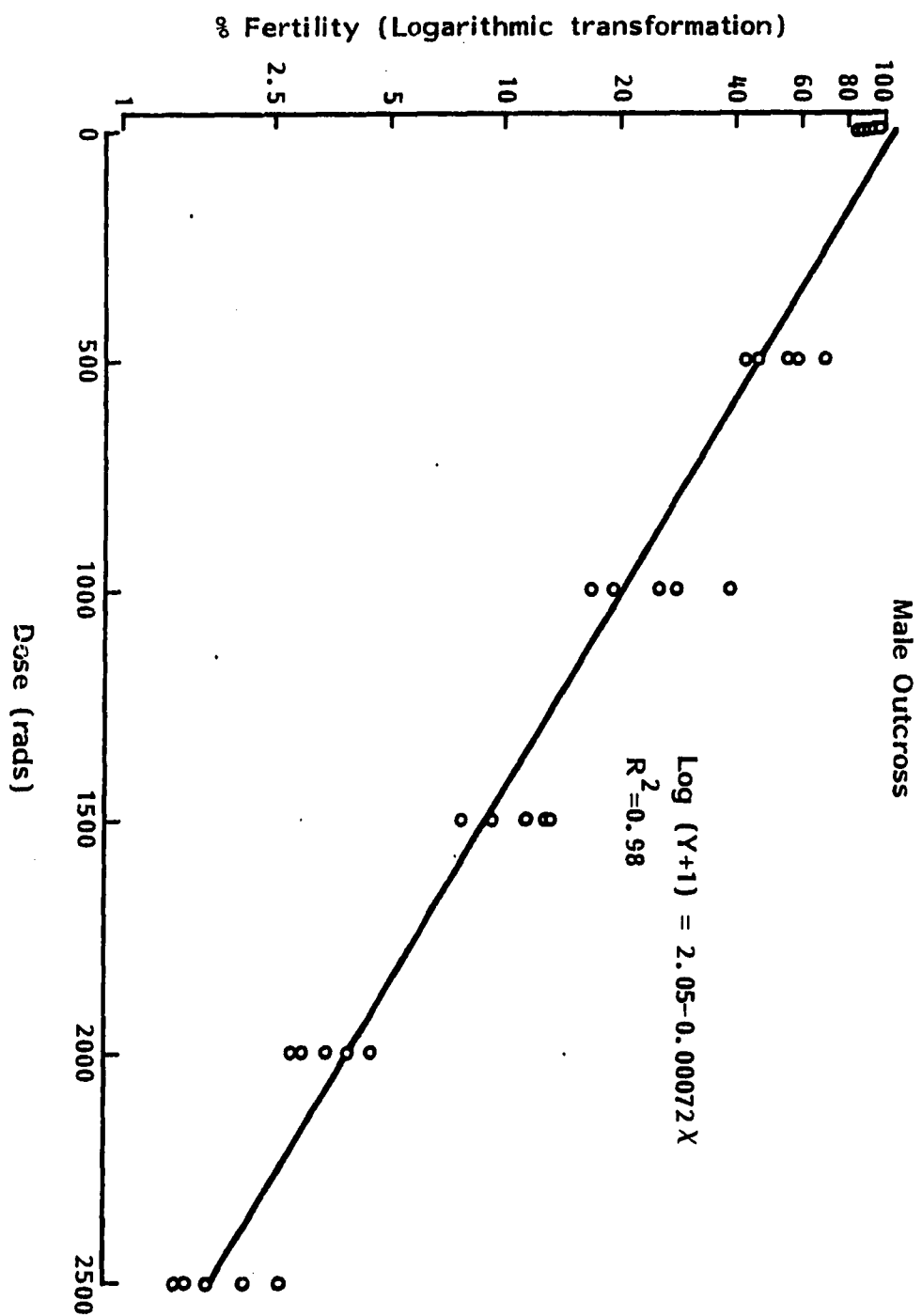


Figure 2. The relationship between gamma radiation dose and male face fly fertility

coefficient ( $R = -.99$ ,  $p = .001$ ). The regression equation predicts the  $LD_{50}$  and the  $LD_{100}$  to be 500 and 2832 rads, respectively. A comparison of  $LD_{100}$  values in face flies, even when irradiated under anoxic conditions, with those of some other diptera (Table 6) shows the high sensitivity of face fly males to ionizing radiation.

Female fertility Female fertility was estimated from percentage egg hatch, pupation and pupal emergence of irradiated females mated to normal males (Table 7). Table 7 shows that increasing radiation dosages resulted in a consistent decrease in egg hatch as well as in pupation and pupal emergence. It also caused a consistent decrease in percentage pupation and pupal emergence from the hatched eggs and pupated larvae.

Figure 3 shows a linear relationship between radiation dose and log fertility ( $y + 1$ ). The  $LD_{50}$  and  $LD_{100}$  predicted by the regression equation, 534 and 2983 respectively, indicates that female face flies are very similar to males in their sensitivity to ionizing radiation.

Percentage of females inseminated The results of the previous experiments indicated that irradiation produced sterility in male and female face flies. However, the results did not suggest what factors might be contributing to the sterility. A study of the spermathecae of 10 of the untreated females outcrossed with treated males in each replicate was, therefore, conducted. The percentage of females inseminated is shown in Table 8. The results indicate that there were no significant differences in percentage of females inseminated by irradiated males. The null hypothesis is supported by the ANOVA (Table 9).

Table 6. Estimated doses of gamma rays required to sterilize males of dipteran species irradiated as pupae<sup>a</sup>

Common and Scientific Name	Sterilizing Dose (r)	Reference
Face fly <u>Musca autumnalis</u> DeGeer	2,100	Mansour (1948)
Screwworm fly <u>Cochliomyia hominivorax</u> (Coquerel)	2,500	Bushland and Hopkins (1953)
Old world screwworm <u>Crysomya bezziana</u> Villeneuve	4,000	Spradbery et al. (1983)
House fly <u>Musca domestica</u> L.	3,000	Sacca (1961)
Stable fly <u>Stomoxys calcitrans</u> (L.)	4,000	Offori (1970)
Horn fly <u>Haematobia irritans</u> (L.)	5,000	Lewis and Eddy (1964)
Oriental fruit fly <u>Dacus dorsalis</u> Hendel	10,000	Steiner et al. (1965)
Melon fly <u>Dacus cucurbitae</u> Coquillett	10,000	Steiner et al. (1965)
Olive fruit fly <u>Dacus oleae</u> (Gmelin)	12,000	Melis and Baccetti (1960)

<sup>a</sup>These studies were done under normal atmospheric conditions (no N<sub>2</sub> was used).

Table 7. The effects of gamma radiation under anoxic conditions on female face fly fertility<sup>a</sup>

Radiation Dose in Rads	The criteria used for measuring sterility						
	% Egg Hatch		% Pupation		% Pupal Emergence		
	Total Number of eggs laid	Total Number of eggs hatched	% Hatch	Total Number of pupae collected	% Pupation	Total Number of pupae eclosed	% Emergence
0	2178	1976	91.7	1784	81.9	1652	75.8
500	2376	1365	57.5	1214	51.1	1100	46.3
1000	2403	692	28.8	576	24.0	489	20.4
1500	2115	273	12.9	206	9.7	158	7.5
2000	2214	75	3.4	33	1.5	17	.8
2500	2193	21	1.0	0	0	0	0

<sup>a</sup>Data are the sum of five replicates.

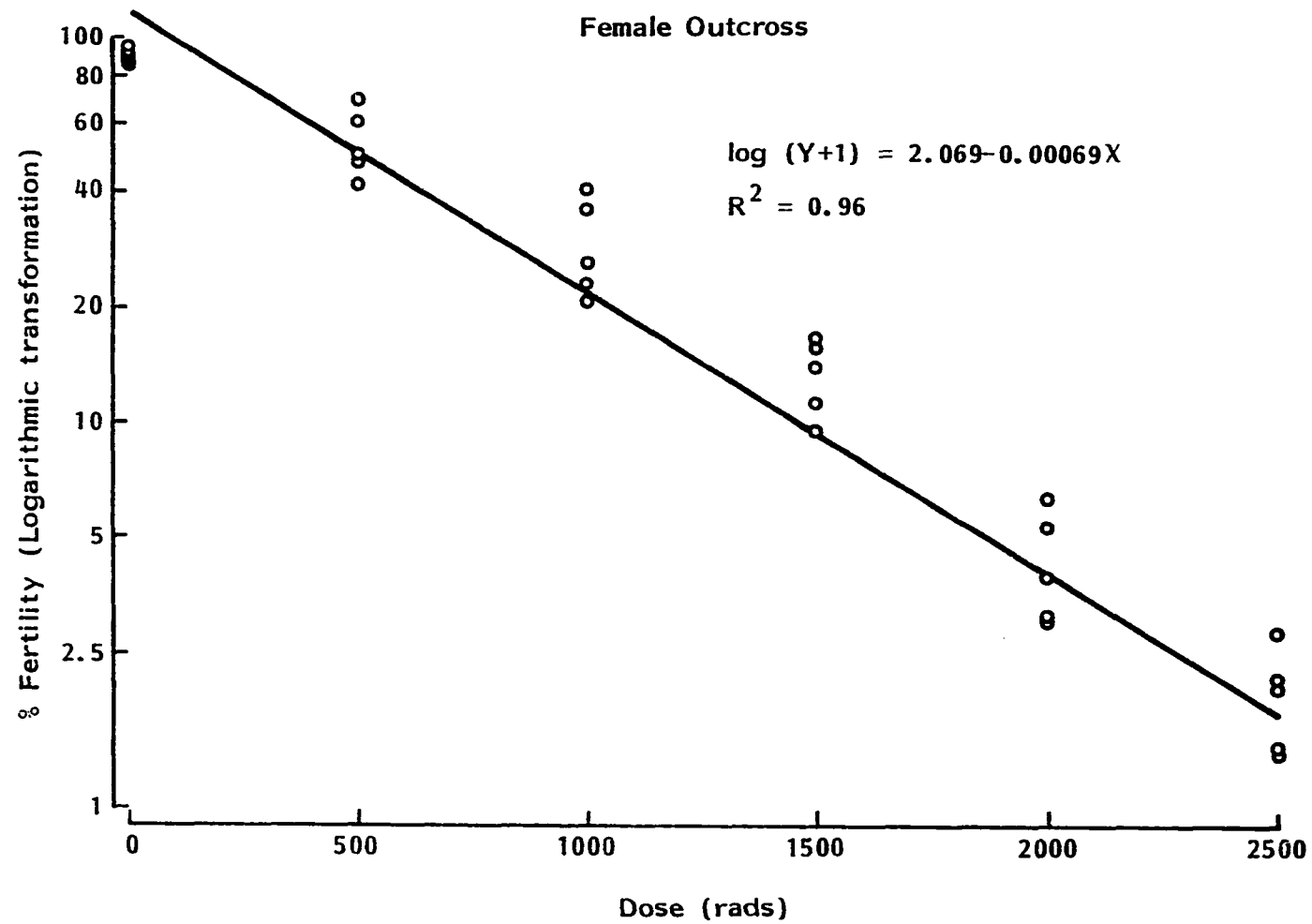


Figure 3. The relationship between gamma radiation dose and female face fly fertility

Table 8. Insemination of face fly females mated with treated males<sup>a</sup>

	Dose (rads)					
	0	500	1000	1500	2000	2500
Females Inseminated (%)	96	94	94	90	92	94

<sup>a</sup>Mean of five replicates, 10 females per replicate.

Table 9. Randomized block analysis of variance to determine the effect of gamma radiation on the ability of treated males to inseminate normal females

Source of Variation	D.f	Sum of squares	Mean squares	F	P
Replicate	4	2.34	.59	1.64	>0.10
Treatment	5	1.07	.21	.58	>0.25
Error	20	7.26	.36		
Total	29	10.67			

Examination of the spermathecae indicated that irradiation did not affect the ability of irradiated males to inseminate normal females. Motility among the spermatozoa was observed in spermathecae of females mated to irradiated males. This suggests that male sterility was not a result of aspermia, lack of sperm motility, or inability of treated males to inseminate females, but rather to dominant lethal mutations.

#### The Permanence of Male Sterility

Previous experiments demonstrated that exposing face flies to 2500 rads in nitrogen as 6-day old pupae reduced the fertility of both males and females to about 1% and completely prevented pupation. This experiment was conducted to determine if there was a recovery of fertility in either males or females treated to 2500 rads. Males were repeatedly exposed to virgin females over a period of 21 days; and conversely, females were repeatedly exposed to virgin males.

The results are shown in Table 10. No improvement was observed in the fertility of unirradiated females mated to increasingly older irradiated males, or treated females mated to untreated males.

#### Effects of Gamma Radiation Under Anoxic Conditions on Male Longevity

To determine the effects of gamma radiation on male longevity, survival of the irradiated males was recorded every other day. The data were tabulated as percentage survival at day 0, 4, 8, 12, 16, 20 and 24 (Table 11).

The data indicate that radiation doses up to 2000 rads had no adverse effect on male survival. However, at 2500 rads mortality was higher than



Table 10. The effects of gamma radiation under anoxic conditions on face fly fertility at 7, 14, and 21 days<sup>a</sup>

Mating type	Number of females	7 days	Number of females	8-14 days	Number of females	15-21 days
NM x NF	300	901	274	789	240	674
Number of eggs laid						
Number of eggs hatched		818		728		621
% Hatch		90.6		92.3		92.1
RM x NF	300	923	269	758	248	688
Number of eggs laid						
Number of eggs hatched		8		7		5
% Hatch		.87		.9		.7
NM x RF	300	937	278	737	238	505
Number of eggs laid						
Number of eggs hatched		11		10		5
% Hatch		1.2		1.4		1.0

<sup>a</sup>Data are the sum of four replicates; 75 males and 75 females were used in each replicate.

<sup>b</sup>Where N (normal), R (irradiated), M (male) and F (female).

Table 11. Percentage survival of irradiated male face flies at 4, 8, 12, 16, 20, and 24 days

Radiation dose in rads	Number of males at day 0	Male Survival <sup>a</sup> (%)					
		4 days	8 days	12 days	16 days	20 days	24 days
0	375	94.4	90.7	84.8	79.2	69.1	60.5
500	375	92.3	88.0	84.0	80.3	66.1	56.8
1000	375	92.8	90.1	85.3	79.5	70.7	56.5
1500	375	93.9	89.9	86.4	81.3	72.3	58.0
2000	375	96.0	91.7	87.7	80.0	69.3	56.0
2500	375	93.3	89.3	85.6	76.8	60.3	42.7

<sup>a</sup>Data are the sum of five replicates.

the control, but only after day 12. The ANOVA (Table 12) shows that both treatment and treatment time interaction were significant. Using the least significant difference (LSD) method for comparing the means at the different dates and dose levels showed that males treated with 2500 rads had significantly lower survival after 12 days of age than control males.

#### Effects of Gamma Radiation Under Anoxic Conditions on Male Competitiveness

In this experiment, egg-hatch data from the mating of normal females with various ratios of irradiated males to normal males were obtained. The following ratios of irradiated males:normal males:normal females were examined: 0:1:1, 1:0:1, 1:1:1, and 9:1:1. The results of this experiment

Table 12. Split-plot analysis of variance to determine the effects of gamma radiation on male survival of face flies

Source of variation	D.f	Sum of squares	Mean squares	F
Replicate	4	111.08	27.77	3.32*
Dose	5	327.30	65.46	7.84**
2500 vs others	1	279.80	279.80	33.50**
ERROR (a)	20	167.04	8.35	
Time	6	24793.83	4132.31	900.29**
time linear	1	23373.53	23373.53	5092.27**
Time * Dose	30	593.77	19.79	4.31**
slope 2500 vs others	1	348.0	348.02	75.85**
ERROR (b)	144	660.69	4.59	
Total	209	26653.70		

\*Significant at the 5% level of probability.

\*\*Significant at the 1% level of probability.

are shown in Table 13. The data indicate that irradiated males were equal in competitiveness to normal males in the ratio 1:1:1 and, more competitive than untreated males when tested in the ratio 9:1:1. To test this hypothesis, the data in Table 13 were subjected to  $\chi^2$  tests (Table 14). The results show that the observed number of hatched larvae was not different from the expected value when a ratio of 1:1:1 was used ( $\chi^2 = 3.78$ ). However, when a ratio of 9:1:1 was used, the trend of competitiveness was in favor of treated males ( $\chi^2 = 8.74$ ).

#### Studies on the Mating Habits of the Female Face Fly

The results of these experiments are shown in Table 15 and 16. Table 15 shows percentage fertility of females mated with treated males and then given the chance to mate with fertile males. The results show a slight improvement in percentage egg hatch (1%). This indicates that a very low percentage of females remated with normal males.

Table 16 shows the mating frequency of labeled females confined with equal numbers of males. The results show that 7.5% of the females remated after the first mating.

Table 13. The effects of gamma radiation under anoxic conditions upon mating competitiveness in face fly males<sup>a</sup>

Ratio <sup>b</sup>	Total number of flies used	Total number of eggs laid	Total number of eggs hatched	% Hatch
0:1:1	630	1897	1707	90.0
1:0:1	630	1828	12	0.7
1:1:1	630	1415	659	46.6
9:1:1	630	442	25	5.7

<sup>a</sup>Data summarize three replicates.

<sup>b</sup>Irradiated male:Untreated male:Untreated female.

Table 14. Chi-square test of results obtained in studies of mating competitiveness tests in face fly males

Ratio <sup>a</sup>	Replicate	Observed (O)	Expected (E)	O-E	$\frac{(O-E)^2}{E}$
1:1:1	1	216	205.2	10.8	.568
	2	236	215.0	21	2.051
	3	207	223.1	-16.1	1.162
					$\chi^2 = 3.78$
9:1:1	1	9	12.2	-3.2	.84
	2	5	15.0	-10	6.7
	3	11	15.3	-4.3	1.2
					$\chi^2 = 8.74^*$

<sup>a</sup>Irradiated male:Untreated male:Untreated female.

\*Significant at the 5% level of probability.

Table 15. Fertility of face fly females mated with sterile males, then given the chance to mate again with normal males<sup>a</sup>

The time for measuring fertility	Number of eggs laid	Number of eggs hatched	% hatch
After mating with sterile males	923	9	.97
After confining the same females with normal males for 6 days	686	18	1.7

<sup>a</sup>Data sum a total of four replicates.

Table 16. Mating frequency in face fly females<sup>a</sup>

Number of females used in the experiment	Number of females mated one time	Number of females mated two times	% females mated after the first mating
40	37	3	7.5

<sup>a</sup>Data sum a total of four replicates.

## DISCUSSION

The objective of these investigations was to determine if radiosterilization of face flies under anoxic conditions could produce males which would be of practical value in a sterile male release program. The results obtained are promising in this regard.

Studies on radiation effects on pupal emergence showed that none of the tested dosages adversely affected adult eclosion. The relative resistance of the 6-day old pupae (one day before emergence) to ionizing radiation is probably due to their age when irradiated. The increase in pupal resistance to ionizing radiation with age has been described before (Ramsamy, 1977; Nair, 1962; Bushland and Hopkins, 1953) and the presence of nitrogen during irradiation might also have had some protective effect (Baumhover, 1963; Smittle, 1967).

In the fertility studies, it is clear that egg hatch, as well as pupation and pupal emergence, decreased with increasing radiation dose. A dose of 2500 rads reduced egg hatch to about 1% in both males and females, and caused 100% mortality in the larvae which were able to hatch (i.e., no pupae were collected from these treatments). This is probably due to the expression of dominant lethal mutations in the larval stage (Borkovec, 1966; Smittle, 1967). However, natural mortality is another factor which should be considered. Tables 5 and 7 show a consistent decrease in percentage pupation and pupal emergence with increasing radiation dose of those larvae which were able to hatch.

The relationship between radiation dose and sterility response, expressed as percentage egg hatch, show that fertility decreases linearly

with dose up to a certain point, after which the curve starts to level off. An explanation of the curvilinear relationship between radiation dose and sterility response requires some background information on the mechanism of inducing dominant lethal mutations by radiation.

Radiation induced dominant lethal mutations arise mainly as a result of chromosome breaks (Muller, 1940; Lea, 1955). Several types of chromosome changes induced by ionizing radiation can be lethal. For instance, a simple chromosome break could be lethal if the break did not rejoin prior to cell division. In addition, certain chromosome breaks which rejoin, but not in the original fashion, also have a dominant lethal effect. These asymmetrical exchanges result in acentric fragments and dicentric chromosomes, which in turn cause genetic imbalance during cleavage division and death of the zygote.

The curvilinear shape of the curve relating dominant lethal mutations to radiation dose can be explained on the basis of one-hit versus multi-hit theory for inducing dominant lethals. The one-hit versus multi-hit idea refers to the number of breaks a chromosome may suffer as a result of exposure to ionizing radiation. Muller (1954) showed that the frequency of individual chromosome breaks in Drosophila melanogaster is linearly proportional to dose. Consequently, the linear portion of the curve at low doses can be explained as being due to single or few chromosome breaks. At higher doses, however, the slope diminishes and the curve levels off, indicating that the frequency of chromosome breaks per gamete are more numerous. As a result, at high doses there is only a small reduction in fertility with increasing dose. This is because there is a high



probability that a single chromosome will suffer multi-hits or several breaks, and since a single break is enough to produce dominant lethal mutation in the zygote at low doses, multi-hits at high doses are superfluous and the curve levels off.

Establishing the dose-response curve for a particular pest species has great practical value. For instance, by establishing this relationship the investigator can predict the dose which produces the highest level of sterility without reducing the biological fitness of the sterilized individuals. This is usually done by determining the point at which the curve starts to level off. Beyond this point, increasing the dose will produce only a small increase in sterility while it may cause reduction in vigor, longevity and competitiveness. In some species it may be more advantageous to release males which are not completely sterile than releasing completely sterile males that have little biological capability.

Longevity studies showed that males irradiated with 2500 rads died a little sooner than unirradiated males. However, it should be noted that the reduced longevity, especially after 16 days of age, might not be a disadvantage in all circumstances. Controlling insect pests by the sterile insect release method requires the release of tremendous numbers of sterilized insects to overwhelm the natural population. In some cases, as in the face fly, the released flies themselves might be a nuisance. However, if their longevity were reduced without adversely affecting mating competitiveness, then the nuisance associated with the release would also be reduced.

In this study, males and females exposed to 2500 rads showed no recovery of fertility within 22 days after irradiation.

Competition studies under laboratory conditions indicated that sterilized males were as competitive as untreated males in inseminating normal females in the ratio 1:1:1 (treated males:untreated males:untreated females respectively) and more competitive than untreated males when tested in the ratio 9:1:1. At this ratio (although most of the deviations came from one replicate) all the replicates showed that the expected number of larvae was greater than the observed number. Treated males in this ratio were, therefore, considered more competitive than untreated ones. However, it is still necessary to study the competitiveness of sterilized males with wild flies under field conditions. Competition studies under field conditions are necessary in order to determine if the wild females would prefer wild males or perhaps would not mate at all with the irradiated males.

Studies on female mating habits showed that only a low percentage of the females displayed polyandry. Although female monogamy is not a prerequisite for the success of the sterile insect release method because competition is not only between males but also between sperms of irradiated and normal individuals, it is important in those cases where the sperms transferred by sterilized males are not as motile as those produced by normal flies. Although it is not clear why some females mate more than once since they gain little but other sperms, it might be possible that those attempting to mate again did not receive a full complement of sperm at the first mating.

After examining the data accumulated during my studies, one might conclude that adult face flies irradiated as 6-day old pupae in a nitrogenous atmosphere could be of practical value in sterile male release programs for control or eradication of natural face fly populations.

Knipling (1955) considered three types of circumstances under which a sterile male release can be considered as a control measure. First, a sterile male release program might be used for control of newly established insect populations before the population density becomes too high. Second, it might be practical for control of important pests that are normally present in small numbers. Third, the sterile male release technique might serve as an adjunct to other control measures when the target species is abundant and widely distributed.

Although face fly natural populations are very low in the early spring when the first summer generation starts to emerge, the high mobility and wide distribution of the face fly in the United States, makes this pest difficult to control with only the sterile male release method. However, using this technique in combination with some others in an integrated pest management program might be very useful in controlling or eradicating face fly populations. An integrated control program utilizing cultural practices, sanitation, biological control agents, insect growth regulators, chemosterilants, and insecticides in the fall might reduce the face fly populations to a very low level that can then be easily flooded with sterilized males the next spring; as a result, an eradication program might be feasible. In fact, sterile males can be released in late August to early September. In this case irradiated flies should enter diapause and

overwinter with the wild populations in their hibernation sites. Then, if they emerged with their wild cousins the next spring, eradication might be achieved. However, the ability of the sterilized flies to survive low temperatures should first be determined in the laboratory and in field studies before any release program is commenced.

## SUMMARY

The face fly, Musca autumnalis DeGeer, is an economically important pest of cattle and horses (Pickens and Miller, 1980; Bech-Nielson et al., 1982; Shugart et al., 1979). Because of the fly's habit of feeding on the mucous secretions of the eyes and nose of its host, insecticidal control is difficult and often unsatisfactory. The use of sterile insect release to control or eradicate this pest could be an advantageous approach. A previous study of radiosterilization of the face fly showed that exposing 6-day old pupae to 1600 rads of gamma radiation induced 97% sterility in face fly males and completely prevented ovarian development in the females (Mansour, 1984). However, this dose also adversely affected male survival and competitiveness. The ultimate objective of this investigation was to determine if radiosterilization of face fly pupae under anoxic conditions could produce males which would be of more practical value in a sterile male release program.

Six day old face fly pupae, one day before emergence, were irradiated in a nitrogenous atmosphere using gamma radiation from a cobalt 60 source. Six levels of gamma radiation were used: 0, 500, 1000, 1500, 2000 and 2500 rads. The effects of gamma radiation on eclosion, reproduction, and male longevity and competitiveness were determined. Female mating habits under laboratory conditions were also examined.

Irradiation under anoxic conditions did not adversely affect adult eclosion, female fecundity or male competitiveness. Sterility in treated males and females increased as radiation dosage increased. A dose of 2500 rads reduced fertility of males to less than 1% and produced similar

results in the females. Longevity of treated males was not affected by increasing radiation dose up to 2000 rads. However, males receiving 2500 rads died sooner than unirradiated males. Studies on the mating habit of the female face fly showed that most of the females mated only once; however, few of them mated a second time.

The results of this study indicate that face fly males treated as 6-day old pupae with 2500 rads in a nitrogenous atmosphere would apparently be satisfactory for use in a sterile male release program.

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## ACKNOWLEDGEMENTS

I wish to express my sincerest gratitude to the chairman of my supervisory committee, Dr. J. Mutchmor, for his guidance, advice, and generous assistance; to the other members of my committee, Drs. H. Stockdale, J. Coats, M. Gleason, L. Pidigo and K. Shaw, for their critical appraisal of the research and their sound advice; to fellow graduate student Mark Bryan for his statistical assistance. And, finally, a special expression of thanks to my wife, Hind, for patience, understanding, encouragment, and enduring many inconveniences during the course of my study.